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Review

Ecological parameters influencing microbial diversity and stability of traditional sourdough

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ABSTRACT

The quality of some leavened, sourdough baked goods is not always consistent, unless a well propagated sourdough starter culture is used for the dough fermentation. Among the different types of sourdough used, the traditional sourdough has attracted the interest of researchers, mainly because of its large microbial diversity, especially with respect to lactic acid bacteria. Variation in this diversity and the factors that cause it will impact on quality and is the subject of this review.

Sourdough microbial diversity is mainly caused by the following factors: (i) sourdough is obtained through spontaneous, multi-step fermentation; (ii) it is propagated using flour, whose nutrient content may vary according to the batch and to the crop, and which is naturally contaminated by microorganisms; and (iii) it is propagated under peculiar technological parameters, which vary depending on the historical and cultural background and type of baked good. In the population dynamics leading from flour to mature sourdough, lactic acid bacteria (several species of *Lactobacillus* sp., *Leuconostoc* sp., and *Weissella* sp.) and yeasts (mainly *Saccharomyces cerevisiae* and *Candida* sp.) outcompete other microbial groups contaminating flour, and interact with each other at different levels. Ecological parameters qualitatively and quantitatively affecting the dominant sourdough microbiota may be classified into specific technological parameters (e.g., percentage of sourdough used as inoculum, time and temperature of fermentation) and parameters that are not fully controlled by those who manage the propagation of sourdough (e.g., chemical, enzyme and microbial composition of flour).

Although some sourdoughs have been reported to harbour a persistent dominant microbiota, the stability of sourdough ecosystem during time is debated. Indeed, several factors may interfere with the persistence of species and strains associations that are typical of a given sourdough: metabolic adaptability to the stressing conditions of sourdough, nutritional and antagonistic interactions among microorganisms, intrinsic robustness of microorganisms, and existence of a stable house microbiota.

Further studies have to be performed in order to highlight hidden mechanisms underlying the microbial structure and stability of sourdough. The comprehension of such mechanisms would be helpful to assess the most appropriate conditions that allow keeping a given traditional sourdough as a stable microbial ecosystem, thus preserving, during time, the typical traits of the resulting product.

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1. Introduction

Sourdough is a mixture of flour (mainly wheat or rye) and water, fermented with lactic acid bacteria (LAB) and yeasts, which are responsible for its capacity to leaven a dough, while contemporarily and unavoidably acidifying it (De Vuyst and Neysens, 2005; Gobbetti, 1998; Vogel et al., 1999). In the modern bakery technology, sourdough represents an alternative to the use of baker's yeast (although bakers often use a combination of both leavening agents) to manufacture a variety of products such as bread, crackers, snacks, pizza and sweet baked goods, because it offers many advantages over baker's yeast: enhanced flavour (Hansen and Schieberle, 2005), prolonged shelf-life (Chavan and Chavan, 2011), improved dough structure (Arendt et al., 2007) and increased nutritional value (Gobbetti et al., 2013; Poutanen et al., 2009) of the leavened baked good. Although liquid (type II) and dried (type III) sourdoughs, produced at industrial level, are fairly widespread among bakers because they are easy to be used (Brandt, 2007), traditional (type I) sourdough tickles researchers' curiosity mainly for its large microbial diversity (De Vuyst et al., 2009). This feature of traditional sourdough is mainly caused by the use of a spontaneous multi-step fermentation, needed for obtaining a sourdough ("mature" sourdough) with a constant leavening and acidifying capacity (Hammes and Gänzle, 1998), and by the use of back-slopping as a tool (almost) daily applied for propagating sourdough (De Vuyst et al., 2009). For this reason, most of the studies dealing with microbial ecology of sourdough focused on traditional sourdough.

The microbial ecology of cereal fermentation has been reviewed by Hammes et al. (2005). Ecological determinants of sourdough microbiota were examined as part of the review by De Vuyst et al. (2009). Other reviews focused either on general aspects (Chavan and Chavan, 2011) or on features of sourdough other than microbial ecology (Arendt et al., 2011; Moroni et al., 2009; Yao et al., 2013). Since 2009, various studies have significantly advanced our knowledge about the microbial ecology of sourdough fermentations and have inspired the justification for this review. Therefore, the objectives of this review are: (i) to give an overview about how LAB and yeasts become the dominant microbial groups in traditional sourdough and how they interact with each other; (ii) to examine factors affecting microbial diversity of traditional sourdough in a systematical way; and (iii) to discuss about parameters influencing the microbial stability of traditional sourdough.

2. Production of sourdough

Traditional sourdough originates from multiple steps of fermentation. In the first step a dough, usually composed of just flour and water, is spontaneously fermented. Then, the fermented dough is used as inoculum for fermenting newly prepared dough, which, in turn, will be used as inoculum for a subsequent step of fermentation. Additional ingredients, such as grape juice/must, honey, hop, overripe fruit, salt, sugar, vinegar may be used in early fermentation steps, in order to include in the dough immediately available nutrients and pro-technological microorganisms. A protocol for the production of a mature "French style" sourdough is given in Fig. 1 (Onno and Roussel, 1994). The figure also shows the typical trend of cell density of different microbial groups during this process. Apart from the first fermentation, the operation named "back-slopping" (or "refreshment"), consisting in the inoculation of flour and water with an aliquot of previously fermented dough, is repeated before each fermentation step. Back-slopping is also applied later, for propagating mature sourdough over time (De Vuyst et al., 2009).

Dough is a nutrient-rich ecosystem. Complex carbohydrates (starch, above all) are present, but their partial hydrolysis to di-saccharides (maltose, above all) and mono-saccharides (fructose and glucose), by flour and microbial amylases, rapidly takes place. Nitrates, ammonia, and proteins constitute the nitrogen sources for microbial growth. During dough fermentation, proteins are hydrolysed to more easily usable nutrients (peptides and free amino acids, FAA) by flour and microbial proteinases. The values of water activity (a_w), ranging from 0.96 to 0.98, do not limit the growth of the majority of contaminant microorganisms. The pH is sub-acid, although, during dough incubation and as the number of back-slopping steps increases, it tends to become acid (values around 4.0) (Table 1 and Fig. 1). The redox potential gradually decreases during dough formation and incubation from positive to negative values (Hammes et al., 2005). Taking into account the above physic-chemical parameters, sourdough allows LAB and yeast to outgrow other microbial populations (Fig. 1). Specific influences of the sourdough ecosystem and its production on the microbial ecology of fermentation will be discussed in later sections.

3. Microbial dynamics from flour to mature sourdough

At the beginning of the first fermentation, the microbial population of dough reflects that of the flour, consisting of LAB, Gram-positive (e.g., *Bacillus* sp.) and Gram-negative (e.g., *Pseudomonas* sp.) aerobic bacteria, *Enterobacteriaceae*, yeasts and moulds (Fig. 1). Each microbial group is present at cell numbers generally not exceeding 5 log CFU/g (Onno and Roussel, 1994; Rocha and Malcata, 2012; Stolz, 1999). Through bacterial 16S rRNA pyrosequencing, it has been recently found that, before the first fermentation, several bacterial phyla (e.g., *Bacteroidetes*, *Cyanobacteria*, *Firmicutes*, and *Proteobacteria*) occur in the dough. However, the majority of these phyla either indicate the presence of a non-active population in the flours or are outcompeted by *Firmicutes* already after the first fermentation (Ercolini et al., 2013), which is consistent with already-known patterns in the microbial ecology of fermented foods (Humblot and Guyot, 2009; Jeong et al., 2013; Jung et al., 2013). Upon addition of water to flour, redox potential of the dough decreases (Hammes et al., 2005), favouring the growth of facultative anaerobes (*Enterobacteriaceae* and yeasts) and of LAB (Fig. 1), most of which are aerotollerant anaerobes. Because carbohydrate metabolism of LAB is highly adapted to mono- and di-saccharides (Gänzle and Gobbetti, 2013), lactic and acetic acids are produced leading to a decrease of pH of the dough. Such a decrease, usually becoming evident after the second fermentation step, may inhibit the growth of *Enterobacteriaceae*, while it is well tolerated by yeasts. Consequently, as the number of fermentation steps increases, LAB and yeasts become more and more adapted to the environmental conditions of sourdough (Fig. 1), until they dominate the mature sourdough (Hammes and Gänzle, 1998), at numbers ranging from 6 to 9 log CFU/g and from 5 to 8 log CFU/g, respectively (Lattanzi et al., 2013; Minervini et al., 2012a). Actually, time (intended as the consecutive fermentation steps) is the variable that mostly affects the structure of the sourdough microbiota (Rocha and Malcata, 2012; Weckx et al., 2010a). For instance, it has been recently found that a gradual succession between the active populations of *Proteobacteria* and *Firmicutes* occurs from the beginning of the first fermentation to the end of the second fermentation of rye-based